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14. ABSTRACT

The overall objective of this project was quantitatively explain the extraordinary stability of the λ-phage lysogen given that the data on the lysis/lysogeny switch dynamics didn't seem to be sufficient for explanation. Further, we aimed to explain the differential stability of a number of operator mutants constructed by John Little. Further, in all cases, we wished to explain the threshold response of the induction switch to different exposures to UV light. Then, based on this understanding we aimed to re-engineer this switch with different thresholds, cooperativities and stabilities.

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Objective: The overall objective of this project was quantitatively explain the extraordinary stability of the λ-phage lysogen given that the data on the lysis/lysogeny switch dynamics didn't seem to be sufficient for explanation. Further, we aimed to explain the differential stability of a number of operator mutants constructed by John Little. Further, in all cases, we wished to explain the threshold response of the induction switch to different exposures to UV light. Then, based on this understanding we aimed to re-engineer this switch with different thresholds, cooperativities and stabilities.

Approach: John Little made a number of directed mutants that changed the predicted feedback structure, binding affinities/cooperativities, and promoter activities of the lambda lysogen. Robin Osterhout from the Arkin lab interned with John Little and worked with him here during his sabbatical to learn the UV induction experiments and with Michael Laub at the CGR at Harvard and Jay Keasling here at Berkeley to learn the microarray analysis we would use to follow host-phage response. She collected (and is still collecting) time-series data on gene expression of each mutant under different UV conditions. These data, then, form a multidimensional matrix with genes on one axis, λ-mutant on the other, experiment on another and time on the last. Robin and Sergey Plyasunov from the Arkin lab have collaborated with little in developing new models of the induction dynamics. First, we have broken everything in the system into elementary reactions and introduced a new biophysical model of the newly discovered PR/PL looping regulation dynamics. This has allowed us to perform a full stochastic sensitivity analysis of the network to deduce what the key controlling factors are. Transcription factor OFF rates and the looping ON rate are the most important features. We have also adapted an extended a method called transition path sampling used in statistical mechanics to application of the chemical master equation which allows us to accurately estimate the transition probabilities between different stable states of our chemical system. This has allowed us to use our model to calculate the stability of

the lysogen even though spontaneous inductions rates are less the 1 in 10^9.

Accomplishments: We have built detailed models of the induction switch including major revisions to the kinetics of the lambda right promoter and a new model of DNA looping. We have developed a new methodology for accurately estimating the frequency of low-probability events. We have used to model to make predictions about the sensitivity of the lambda switch to different mutations. Experimentally, we have recently gotten the microarray analysis for the E.coli/λ system working sufficiently well to provide the data necessary for testing the model. Wild-type and four different λ mutants have been exposed to differing dosages of UV and a time courses of their response have been collected. The gene expression during this response is being followed now by microarray.

Conclusions: Such quantitative study of even so simple a genetic switch requires the very careful and rigorous application of a number of difficult technologies. While we did not accomplish all our goals within the project period, this grant allowed us to get the pipeline up and running. Now with new simulation technology, new biophysical models, and new experimental techniques in hand, we are in a position to answer all the questions we started out with. Our initial results indicate that the newly discovered PR/PL looping regulation is sufficient to explain all of Prof. Little's mutant results but that there are difficulties in explaining the incredible stability of wild-type. However, we await the full data matrix to compare our models to before we make any final conclusions.

Significance: Apart from developing and hardening a entirely new technology for screening the activity of new molecules and identifying their protein targets—this method is now being adapted to screen for new classes of druggable proteins for use in human therapeutics and for deducing networks of interaction in the yeast cell.

Patent Information: No patents have been filed related to this research

Award Information: 2001, Arkin Elected to the Howard Hughes Medical Institute

Publication and Abstracts:

Two papers are in preparation on the modeling technology developed for this grant—but further publication awaits collection of the data.